Amendments to the Specification

Please replace the paragraph at page 1, lines 4-21 with the following amended paragraph:

This application is a divisional of U.S. Application Serial No. 09/927,703, filed August 10, 2001, which is a continuation of U.S. Application Serial No. 09/756,398, filed January 8, 2001, now U.S. Patent No. 6,835,823, issued December 28, 2004, which is a divisional of U.S. Application Serial No. 09/133,119, filed August 12, 1998, now U.S. Patent No. 6,277,969, which is a divisional of U.S. Application Serial No. 08/570,674, filed December 11, 1995, now abandoned, which is a continuation-in-part of U.S. Application Serial No. 08/324,799, filed October 18, 1994, now U.S. Patent No. 5,698,195, issued December 16, 1997, which is a continuation-in-part of U.S. Application Serial Nos. 08/192,102, now U.S. Patent No. 5,656,272, issued August 12, 1997, 08/192,861, now U.S. Patent No. 5,919,452, issued July 6, 1999, and 08/192,093, now U.S. Patent No. 6,284,471, issued September 4, 2001, all filed on February 4, 1994 which are continuations-in-part of U.S. Application Serial No. 08/010,406, filed January 29, 1993, now abandoned, and U.S. Application Serial No. 08/013,413, filed February 2, 1993, now abandoned, which is a continuation-in-part of U.S. Application Serial No. 07/943,852, filed September 11, 1992, now abandoned, which is a continuation-in-part of U.S. Application Serial No. 07/853,606, filed March 18, 1992, now abandoned, which is a continuation-in-part of U.S. Application Serial No. 07/670,827, filed March 18, 1991, now abandoned. Each of the above applications are entirely incorporated herein by reference.

Please replace the paragraph at page 15, line 21 through page 16, line 3 with the following amended paragraph:

Figures 33A-33H are graphical representations of analyses of binding between the various fusion proteins and TNFα by saturation binding (Figure 33A and 33B) and Scatchard analysis (Figure 33C-33H). A microtiter plate was coated with excess goat anti-Fc polyclonal antibody and incubated with 10 ng/ml of fusion protein in TBST buffer (10 mM Tris-HCl, pH 7.8, 150 mM NaCl, 0.05% ~~Tween-20~~ TWEEN® 20) for 1 hour. Varying amounts of ^{125}I labeled TNFα (specific activity - 34.8 μCi/μg) were then incubated with the captured fusion protein in PBS (10 mM Na Phosphate, pH 7.0, 150 mM NaCl) with 1% bovine serum albumin for 2 hours. Unbound TNFα was washed away with four washes in PBS and the cpm bound was quantitated

using a y-counter. All samples were analyzed in triplicate. The slope of the lines in (Figures 33C-H) represent the affinity constant, K_a. The dissociation constant (K_d) values (see Table 1) were derived using the equation $K_d=I/K_a$.

Please replace the paragraph at page 58, line 1 through page 59, line 14 with the following amended paragraph:

TNF related pathologies include, but are not limited to, the following:

(A) acute and chronic immune and autoimmune pathologies, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, thyroidosis, graft versus host disease, scleroderma, diabetes mellitus, Graves' disease, Beschet's disease, and the like;

(B) infections, including, but not limited to, sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, acute and chronic parasitic and/or infectious diseases, bacterial, viral or fungal, such as a HIV, AIDS (including symptoms of cachexia, autoimmune disorders, AIDS dementia complex and infections);

(C) inflammatory diseases, such as chronic inflammatory pathologies and vascular inflammatory pathologies, including chronic inflammatory pathologies such as sarcoidosis, chronic inflammatory bowel disease, ulcerative colitis, and Crohn's pathology and vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, and Kawasaki's pathology:

(D) neurodegenerative diseases, including, but are not limited to, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranucleo palsy; Cerebellar and Spinocerebellar Disorders, such as astructural lesions of the cerebellum; spinocerebellar degenerations (spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and MachadoJoseph)); and systemic disorders (Refsum's disease, abetalipoprotemia, ataxia, telangiectasia, and mitochondrial multi-system disorder); demyelinating core disorders, such as multiple sclerosis,

acute transverse myelitis; disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy);Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerrorden-Spatz disease; and Dementia pugilistica, or any subset thereof;

(E) malignant pathologies involving TNF-secreting tumors or other malignancies involving TNF, such as, but not limited to leukemias (acute, chronic myelocytic, chronic lymphocytic and/or myelodyspastic syndrome); lymphomas (Hodgkin's and non-Hodgkin's lymphomas, such as malignant lymphomas (Burkitt's lymphoma or Mycosis fungoides)); carcinomas (such as colon carcinoma) and metastases thereof; cancer-related angiogenesis; infantile haemangiomas;

(F) hepatitis, e.g., alcohol-induced hepatitis; and

(G) other diseases related to angiogenesis or VEGF/VPF, such as ocular neovascularization, psoriasis, duodenal ulcers, angiogenesis of the female reproductive tract.

Please replace the table at page 84 with the following amended table:

TABLE 2 *In Vitro* Neutralization of TNF-Induced IL-6 Secretion

		TNF Concentration (ng/ml)		
Antibody	0	0.3	1.5	7.5
None	<0.20	1.36	2.00	2.56
Control mAb	<0.20	1.60	1.96	2.16
cA2	<0.20	<0.20	<0.20	0.30

Values represent mean concentrations of IL-6 of duplicate wells, in ng/ml. RhTNF (Suntory, Osaka, Japan), with or without 4 μg/ml antibody, was added to cultures of FS-4 fibroblasts and after 18 h, the supernatant was assayed for IL-6 using the ~~QUANTIKINE~~ QUANTIKINE®

Human IL-6 Immunoassay (from R&D Systems, Minneapolis, MN). Control mAb = chimeric mouse/human IgG1 anti-platelet mAb (7E3).

Please replace the paragraph at page 89, lines 16 through 19 with the following amended paragraph:

Sodium dihydrogen phosphate (31.2 g, Sigma cat # S-0751 or equivalent) and sodium dodecylsulfate (20.0 g, Sigma cat # L-3771 or equivalent) were dissolved in 2.0 L of ~~milliQ~~ MILLI-Q® water. The pH was adjusted to 7.2 ± 0.1 with 50% w/w sodium hydroxide (VWR cat # VW6730-3 or equivalent).

Please replace the paragraph at page 89, line 21 through page 90, line 3 with the following amended paragraph:

Sodium dihydrogen phosphate (0.39 g, Sigma cat #S-0751 or equivalent) disodium hydrogen phosphate (1.07 g, Baker cat # 3828-1 or equivalent) and sodium chloride (8.50 g, Baker cat # 3624-5 or equivalent) were dissolved in 1.0 L of ~~milliQ~~ MILLI-Q® water. The pH was adjusted to 7.2 ± 0.1 with 50% w/w sodium hydroxide (VWR cat VW6730-3 or equivalent). Chicken egg albumin (10.0 g, Sigma cat #A-5503 or equivalent) and bovine serum albumin (10.0 g, Sigma, cat #A-3294 or equivalent) were dissolved at room temperature with gentle stirring. The solution was filtered, and to the solution was added ~~Tween 20~~ TWEEN® 20 (2.0 ml, Sigma cat #P-13.79 or equivalent). The solution was stirred gently at room temperature for 30 min, filtered and stored at 40°.

Please replace the paragraph at page 90, lines 4 through 11 with the following amended paragraph:

PBS/~~Tween 20~~ TWEEN® 20

A 10 x concentrate was prepared by dissolving sodium dihydrogen phosphate (3.90 g, Sigma cat # S-0751 or equivalent), disodium hydrogen phosphate (10.70 g, Baker cat #3828-1 or equivalent) and sodium chloride (85.0 g, Baker cat #3624-5 or equivalent) in 1.0 L of ~~milliQ~~ MILLI-Q® water. The pH was adjusted to 7.2 ± 0.1 with 50% w/w sodium hydroxide (VWR cat #VW 6730 or equivalent). To the solution was added ~~Tween 20~~ TWEEN® 20 (5.0 mL, Sigma

cat #P-1379 or equivalent), and the mixture stirred gently. Just prior to use 100 mL of this solution was diluted to 1.0 L with ~~milliQ~~ MILLI-Q® water.

Please replace the paragraph at page 90, lines 13 through 19 with the following amended paragraph:

Substrate buffer was prepared by dissolving citric acid (4.20g, Malinckrodt cat #0627 or equivalent) and disodium hydrogen phosphate (7.10 g, Baker cat #3828-1 or equivalent) in 1.0 L of ~~milliQ~~ MILLI-Q® water. The pH was adjusted to 5.00 with 50% w/w sodium hydroxide (VWR cat #VW6730-3 or equivalent). Immediately prior to use an OPD substrate tablet (30 mg, Sigma cat #P-8412 or equivalent and 30% (v/v) hydrogen peroxide (40 µL, Sigma cat #P-1379 or equivalent) were added to the substrate buffer 25.0 mL). The solution was wrapped in foil and mixed thoroughly.

Please replace the paragraph at page 90, lines 21 through 22 with the following amended paragraph:

Sulfuric acid (53 mL, EM Science cat #SX1244-5 or equivalent) was slowly added to ~~MILLI-Q~~ MILLI-Q® water (447 mL) and cooled to room temperature prior to use.

Please replace the paragraph at page 91, lines 4 through 9 with the following amended paragraph:

Prior to use and after each subsequent use the peptide pins were cleaned using the following procedure. Disruption buffer (2.0 L) was heated to 60° and placed in an ultra-sonic bath in a fume hood. To the disruption buffer was added dithiolthreitol (2.5 g, Sigma cat #D-0632 or equivalent). The peptide pins were sonicated in this medium for 30 min, washed thoroughly with ~~milliQ waster~~ MILLI-Q® water, suspended in a boiling ethanol bath for 2 min, and air-dried.

Please replace the paragraph at page 91, lines 10 through 27 with the following amended paragraph:

Blocking buffer (200 μL) was added to a 96 well disposable polystyrene Elisa plate and the peptide pins suspended in the wells. The peptide pins and plate were incubated for 2 hours at room temperature on an oscillating table shaker. The plates and peptide pins were washed with PBS/~~Tween 20~~ TWEEN® 20 (four times). To each well was added a 20 μg/ml concentration of cA2 antibody (diluted with blocking buffer, 175 μL/well). TNF competition was done by incubation of TNFα (40 μg/ml) and cA2 (20 μg/ml) in BSA/ovalbumin/ BBS for three hours at room temperature. The peptide pins were suspended in the plate and incubated at 4° overnight. The peptide pins and plate were washed with PBS/~~Tween 20~~ TWEEN® 20 (four times). To each well was added anti-human goat antibody conjugated to horseradish peroxidase (diluted with blocking buffer to 1/2000, 175 μL/well, Jackson IMMUNORESEARCH Labs). The peptide pins were suspended in the plate, and incubated for 1 hour at room temperature on a oscillating table shaker. The plates and peptide pins were washed with PBS/~~Tween 20~~ TWEEN® 20 (four times). To each well was added freshly prepared substrate solution (150 μL/well), the peptide pins were suspended in the plate and incubated for 1 hour at room temperature on an oscillating table shaker. The peptide pins were removed and to each well is added 4N H_2S0_4 (50 μL). The plates were read in a Molecular Devices plate reader (490 nm, subtracting 650 nm as a blank), and the results are shown in Figures 14A and 14B, as described above.

Please replace the paragraph at page 110, lines 17 through 25 with the following amended paragraph:

This 16 year old patient has a history of Crohn's disease since age 12. She was suffering from diarrhoea, rectal blood loss, abdominal pain, fever and weight loss. She showed perianal lesions, severe colitis and irregularity of the terminal ileum. She was treated with prednisolone (systemic and local) and ~~pentasa~~ PENTASA®. This resulted in remission of the disease, but she experienced extensive side effects of the treatment. She experienced severe exacerbations at age 12 and 12 yrs, 5 months, (~~Immuran™~~ IMMURAN™ added), 12 yrs, 9 months, 13 yrs, 5 months, and 14 yrs, 10 months. She experienced severe side effects (growth retardation, morbus Cushing, anemia, muscle weakness, delayed puberty, not able to visit school).

Please replace Table 11 at pages 112 through 113 with the following amended table:

TABLE 11 Case History SB

11y, 8m	Physical Examination	Diarrhoea, rectal blood loss, abdominal pain, fever (40%) weight loss perianal lesions
	Sigmoidoscopy	Severe colitis, probably M. Crohn
	Enterolysis	Irregularity terminal ileum
	Therapy	Prednisolone 10 mg 3 dd ~~Pentasa~~ PENTASA® 250 mg 3 dd Enema (40 mg prednisone, 2g 5 ASA) ml 1 dd
	Result	Remission, however: extensive side effects of prednisone and stunting growth
	Action	Prednisone
11y, 11m	Exacerbation	Same clinical picture as 11y, 8m
	Sigmoidoscopy	Recurrence of colitis (grade IV) in last 60 cm and anus
	Therapy	Prednisolone 40 mg 1 dd ~~Pentasa~~ PENTASA® 500 mg 3 dd Enema 1 dd
	Result	Better
12y, 5m	Severe Exacerbation	Despite intensive treatment
	Sigmoidoscopy	Extensive perianal and sigmoidal lesions; active disease
	Therapy	Continued + ~~Immuran~~™ IMMURAN™ 25 mg 1 dd
	Result	Slight improvement, however still growth retardation, cushing, anaemia, muscle weakness
	Action	Prednisone

TABLE 11 Continued

12y, 9m	Exacerbation	
	Sigmoidoscopy	Extensive (active colitis, polyps)
	Action	Prednisone: 30 mg 1 dd, ~~Immuran™~~ IMMURAN™ 50 mg 1 dd ~~Pentasa~~ PENTASA® 500 mg 3 dd Enema 2 dd
	Result	Still needs enemas with prednisone and oral prednisone. Delayed puberty, stunting growth
14y, 10m	Severe Exacerbation	Weight loss, abdominal pain, fever
	Ileoscopy	Active colitis (grade IV), perianal lesions. Terminal ileum normal
	Result	No remission still fever, poor appetite, weight loss, diarrhea, not able to visit school
Important Findings		
14y, 11m	151.9 cm; 34 kg; t = 38°C, Abdominal mass in right lower quadrant; stool frequency 28 per week (however goes 10-15 times a day but most often without success); ESR 55 mm; Hb 6.2 mmol/1; Ht 0, 29 l/l; alb. 38.4 g/l Crohn's Dis./Act Index: 311 Pediatric score: 77.5	
14y, 11.2m	151,8 cm; 34.6 kg (before 1st infusion) Crohn's Dis/Act Index: 291 Pediatric score: 60	
14y, 11.4m	151,8 cm; 34.6 kg; ESR 332 mm; Hb 5.7 mmol/l Crohn's Dis/Act Index: 163 Pediatric score: 30	
15y, 0m	152,1 cm: 34.8 kg (before 2nd infusion) Feels like she has never felt before. Parents also very enthusiastic; ESR 30 mm: Hb 6. 3 mol/l; Ht 0, 32 11; Alb 46 g/l Crohn Dis/Act Index: 105 Pediatric Score: 15 Videoendoscopy: Improvement No problems or side effects observed during and following infusion.	

Please replace the paragraph at page 128, lines 22 through 26 with the following amended paragraph:

The patient is a 41 year old woman with long term ulcerative colitis, which was diagnosed by endoscopy and histology. She has a pancolitis, but the main disease activity was left-sided. There were no extra-intestinal complications in the past. Maintenance therapy consisted of ~~Asacol™~~ ASACOL®. Only one severe flair-up occurred 4 years previously and was successfully treated with steroids.

Please replace the paragraph at page 128, line 27 through page 129, line 2 with the following amended paragraph:

At beginning month one, she was admitted elsewhere because of a very severe flair-up of the ulcerative colitis. Treatment consisted of high doses of steroids intravenously, antibiotics, ~~asacol~~ ASACOL® and Total Parental Nutrition. Her clinical condition worsened and a colectomy was considered.

Please replace the paragraph at page 129, lines 6 through 10 with the following amended paragraph:

Medication: ~~ASACOL~~ ASACOL® 2 dd 500 mg, orally
Di-Adresone-T 1 dd 100--mg, intravenously
Flagyl 3 dd 500 mg, intravenously
Fortum 3 dd 1 gram, intravenously
Total parental nutrition via central venous catheter

Please replace the paragraph at page 153, lines 2 through 13 with the following amended paragraph:

A comparison was made of the binding affinity of various fusion proteins and TNFα by saturation binding (Figures 33A and 33B) and Scatchard analysis (Figures 33C-33H). A microtiter plate was coated with excess goat anti-Fc polyclonal antibody and incubated with

10 ng/ml of fusion protein in TBST buffer (10 mM Tris-HCl, pH 7.8, 150 NaCl, 0.05% ~~Tween-20~~ TWEEN® 20) for 1 hour. Varying amounts of ^{125}I labeled TNFα (specific activity - 34.8 μCi/μg) was then incubated with the captured fusion protein in PBS (10 mM Na Phosphate, pH 7.0, 150 mM NaCl) with 1% bovine serum albumin for 2 hours. Unbound TNFα was washed away with four washes in PBS and the cpm bound was quantitated using a y-counter. All samples were analyzed in triplicate. The slope of the lines in (Figures 33C-H) represent the affinity constant, K_a. The dissociation constant (K_d) values (see Table 1) were derived using the equation $K_d = 1/K$.